Prevalence of failure of passive transfer of immunity in newborn heifer calves and associated management practices on US dairy operations

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ABSTRACT

Failure of passive transfer of immunity (FPT) in dairy replacement calves has been linked to increased neonatal morbidity and mortality and long-term decreases in productivity. The purpose of this study was to estimate the prevalence of FPT in US dairy heifer calves in 2007 and to use nationally representative data to investigate associations of FPT with colostrum and calf management practices. A cross-sectional study was conducted by the USDA's National Animal Health Monitoring System between January and August 2007. Producers from 394 operations in 17 states completed survey questions about colostrum and calf management practices, and serum samples were collected from 1,816 healthy heifer calves on those operations. Serum immunoglobulin G (IgG) levels were determined by radial immunodiffusion, and calves were classified as having FPT if the IgG concentration was less than 10 mg/ mL. To investigate associations between FPT and management practices, a multivariable analysis was completed using a weighted logistic regression model. The estimated prevalence of FPT in US dairy heifer calves was 19.2%. The odds of FPT were higher for calves on operations that pooled colostrum [odds ratio (OR = (OR = 2.4), or hand fed colostrum more than 4 h after birth (OR = 2.7). The odds of FPT were also higher for calves on operations that did not provide a source of heat during cold weather for calves experiencing a dystocia (OR = 1.6), would not seek veterinary assistance when unable to correctly position a calf for delivery (OR = 2.6), or did not routinely monitor serum proteins in calves as a measure of passive transfer (OR = 13.8). The prevalence of FPT in dairy heifer calves has decreased in the last 15 yr, so progress has been made in this important area of calf management. This study identified several management practices associated with FPT that could be targeted for educational campaigns or further research.

Key words: dairy calf, failure of passive transfer of immunity, risk factor, immunoglobulin G

INTRODUCTION

Because calves are born nearly agammaglobulinemic, they depend upon ingestion of colostrum to obtain immunoglobulins for protection against infectious diseases. A calf's gastrointestinal tract is designed to temporarily allow the absorption of large molecules, including immunoglobulins, during the first 12 to 24 h of life. Although colostrum contains several types of immunoglobulins (IgG, IgA, IgM), IgG constitutes approximately 85% of the immunoglobulins in colostrum (Butler, 1983). Failure of passive transfer of immunity (FPT) occurs when a calf fails to absorb an adequate quantity of immunoglobulin. Based on previous research, there are 4 key factors that contribute to successful passive transfer of immunity: feeding colostrum with a high immunoglobulin concentration (>50 mg/ mL of IgG), feeding an adequate volume of colostrum, feeding colostrum promptly after birth, and minimizing bacterial contamination of colostrum (Weaver et al., 2000; Johnson et al., 2007; Godden, 2008).

Successful passive transfer is important to dairy producers for several reasons. Failure of passive transfer has been linked with increased calf morbidity and mortality and a reduction in calf growth rate (Robison et al., 1988; Wells et al., 1996; Donovan et al., 1998). In addition, FPT in heifer calves affects long-term productivity—low IgG levels have been associated with decreased first- and second-lactation milk production and an increased culling rate during the first lactation (DeNise et al., 1989; Faber et al., 2005).

Measurement of calf serum IgG level by radial immunodiffusion (RID) is the standard for determining the level of passive transfer of immunity. Passive transfer is considered adequate if the IgG level is 10 mg/mL (1,000 mg/dL) or greater at 1 to 7 d of age (Tyler et al., 1996; BAMN, 2001). In 1991–1992, the National Dairy Heifer Evaluation Project estimated that over 40% of dairy heifer calves had FPT based on IgG levels (USDA, 1993); consequently, this area has been the

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target of educational campaigns (BAMN, 2001). An updated population estimate for the prevalence of FPT in dairy calves would be useful to evaluate the progress made in colostrum and calf management.

The purpose of this study was to estimate the prevalence of FPT in US dairy heifer calves in 2007, and to use nationally representative data to investigate associations of FPT with colostrum and calf management practices.

MATERIALS AND METHODS

Study Design

A cross-sectional study was conducted by the USDA's National Animal Health Monitoring System (NAHMS) between January and August 2007. Data were collected from dairy operations in 17 states that represented 82.5% of US dairy cows and 84.7% of US dairy operations with 30 or more milk cows. The states and regions in the study were designated East (Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin) and West (California, Idaho, New Mexico, Texas, and Washington). The survey design was a stratified random sample with unequal selection probabilities. Selection probabilities were unequal to ensure the inclusion of large dairy operations. All respondent data were statistically weighted to ensure that samples reflected the subject population. Complete details of the sample weighting and the study design are available (USDA, 2007).

Data Collection

The first phase of the study involved a questionnaire about general management practices on farms with one or more dairy cows present on January 1, 2007. Farms that participated in phase I and had 30 or more dairy cows were eligible to participate in phase II. Phase II included 2 additional questionnaires on management practices. Data from phase I and phase II questionnaires are referred to as "herd-level variables" in this report. Enumerators from the National Agricultural Statistics Service collected data in phase I, whereas federal and state veterinary medical officers and animal health technicians collected questionnaire data during phase II.

Operations that participated in phase II were given the opportunity to have serum from newborn heifer calves tested for IgG levels. Between February and August 2007, USDA: Animal and Plant Health Inspection Service: Veterinary Services (USDA:APHIS:VS) and state personnel collected blood from a maximum

of 10 calves per operation. Personnel were instructed to collect blood from healthy heifer calves between 1 and 7 d of age that had received colostrum. For each calf tested, information was recorded about the calf's age at the time of blood collection, the quantity of colostrum the calf received at first feeding, and the method by which colostrum had been administered. These data are referred to as "calf-level variables" in this report. A total of 2,030 blood samples were collected from dairy calves on 413 operations in 17 states. Blood samples were shipped on ice to the National Veterinary Services Laboratories in Ames, Iowa. Blood samples were received in serum separator tubes and centrifuged to separate the serum. The sample tubes were stored refrigerated at 4° C for up to 5 d and then stored at -20° C until tested. All serum samples were tested over a period of 16 d using a commercially available RID kit (Bovine IgG SRID Kit, VMRD, Pullman, WA). The kit has an IgG detection range of 400 to 3,200 mg/dL (4 to 32 mg/mL). Briefly, 3 µL of each of 4 reference standards was placed into the first 4 wells of a plate from each kit. For each sample tested, 3 µL of serum was placed into a well of one plate from the kit. The plates were covered and left at room temperature for 18 to 21 h. Subsequently, the diameters of the rings (in mm) were read using a Finescale comparator (Finescale, Orange, CA) and a standard curve established. The IgG concentration of each sample was determined by finding the point on the standard curve that corresponded to the sample's ring diameter and then determining the immunoglobulin concentration that corresponded with that point. Samples with diameters that were too small to read were classified as <4 mg/mL and those with diameters too large to read were classified as >32 mg/ mL. Calves were classified as having FPT if the IgG level was <10 mg/mL.

Samples from calves that were <1 d of age or >7 d, were bull calves, were unhealthy, or had received a colostrum supplement or replacer were excluded from analysis. A total of 214 samples were excluded for these reasons, resulting in statistical analysis being performed on 1,816 samples from 394 operations in 17 states.

Statistical Analysis

Statistical analyses incorporated the weights to allow the sample to reflect the population from which it was selected (calves on US dairy operations with 30 or more dairy cows in the 17 states included in this study). A commercially available software program (SUDAAN, Release 8, Research Triangle Institute, Research Triangle Park, NC) was used, which accounted for the complex survey design, including the weights, and nesting of calves within farms.

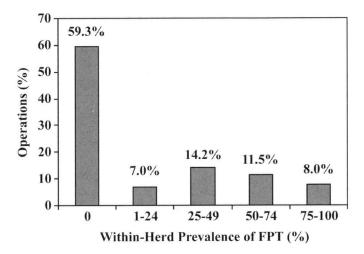


Figure 1. Within-herd prevalence of failure of passive transfer (FPT) in heifer calves on US dairies.

Univariable Analysis of Herd-Level and Calf-Level Variables

The associations between FPT and 18 independent variables were initially screened using chi-square tests. Independent variables included herd-level management practices and calf-level variables. Serum IgG values were grouped into 2 levels based on our selected definition of FPT (<10 and $\geq \! 10$ mg /mL) and used as the outcome variable.

Multivariable Analysis of Herd-Level Variables

To examine more complex associations between the outcome and multiple independent variables, a multivariable analysis of herd-level variables was completed using a weighted logistic regression analysis. Herd-level independent variables associated (P < 0.3) with FPT in the screening analysis were included in the multivariable analysis. Herd size and region were offered to the model even though they did not meet the screening criteria. A backward elimination procedure was used to create the final model, and variables with Wald F P-value < 0.05 were considered statistically significant. Logical 2-way interactions were evaluated.

For this study, producers who generally allowed calves to nurse were not asked about the usual time of first colostrum feeding because they were not expected to know this information. Therefore, all of the calves that nursed had "missing" data for this variable. To avoid excluding all the calves that nursed from the multivariable analysis, nursing was introduced as a level for this variable. Consequently, the categories for the usual time of first colostrum feeding were ≤ 4 h after birth, >4 h after birth, and unknown (allowed to nurse).

Multivariable Analysis of Calf-Level Variables for Calves Hand-Fed Colostrum

A multivariable analysis of calf-level variables was also completed using a weighted logistic regression model. Nursing calves were excluded from this model because the association between nursing and FPT was already examined in the herd-level multivariable analysis. Calf-level variables associated (P < 0.3) with FPT in the screening analysis were offered for inclusion in the multivariable model. Herd size and region were also offered for inclusion in the model. A backward elimination procedure was used to create the final model and variables with Wald F P-value < 0.05 were considered statistically significant.

RESULTS

Prevalence of FPT

Serum IgG levels in heifer calves ranged from <4 to >32 mg/mL (Table 1). Overall, 19.2% of calves had FPT and 25.4% of calves had IgG levels of 32 mg/mL or greater. Additionally, 40.7% of operations had at least one calf with FPT (Figure 1).

Herd-Level Management Practices and Univariable Associations

Based on questionnaire responses about usual management practices, a very low percentage of calves were on operations that pasteurized colostrum (0.8%) or routinely monitored serum proteins (3.3%; Table 2). About 1 in 5 calves (19.5%) were on operations that pooled colostrum from more than one cow.

The herd-level variables associated (P < 0.3) with FPT in the univariable analysis were whether or not the operation routinely monitored serum protein in heifer calves (P = 0.001), usual time of first colostrum feeding on the operation (P = 0.002), whether or not the operation would seek veterinary assistance when

Table 1. Distribution of radial immunodiffusion IgG results from dairy heifer calves 1 to 7 d of age on US dairies

	Ca	lves
IgG level (mg/mL)	%	SE
<4	6.3	1.0
4 to <10	12.9	1.3
10 to <15	14.1	1.3
15 to <20	12.6	1.1
20 to < 25	15.1	1.4
25 to < 32	13.6	1.2
>32	25.4	2.0
Total	100.0	

on passive transfer of of of of herd-level variables for t practices, 8 l-level management p Jo Table 2. Percentage US dairies

			Percentage serum I	Percentage of calves by serum IgG level	
Herd-level variable	revel sales	Calves (%)	≥10 mg/mL (adequate)	<10 mg/mL (failure)	χ^2 <i>P</i> -value
All calves	TIS STATE	100.0	80.8	19.2	
Kegion	East	83.7	81.2	18.8	0.635
Herd size (number of cows)	West	16.3	78.8	21.2	
	100 to 499	32.6	80.5 82.8	19.5	0.587
	>500	16.2	77.9	22.1	
Usual time of first colostrum feeding	Unknown (allowed to nurse)	26.2	76.4	23.6	0.002
	>4 h after birth	15.1	70.8	29.2	
Pools colostrum	Yes	19.5	5.60	23.1	0.973
	No	80.5	82.1	17.9	0.17
Stores excess colostrum	Yes	58.5	84.4	15.6	0.343
	No	41.5	9.08	19.4	
r asceurizes coloserum	Yes	0.8	70.4	29.6	0.464
Routinely monitors comm metains in newbown beits and	ONI	99.2	81.2	18.8	
control moreous set uni proteins in new born nener carves	Yes	က က (97.2	2.8	0.001
Has a system for scoring calving difficulty	NO NY	7.96.7	80.3	19.7	
we assess to seeming carving uniformly	res	45.0	000 000 000 000 000 000 000 00	16.2	0.093
Seeks veterinary assistance for difficult deliveries when unable to correctly	Yes	93.3	82.5	21.4	900 0
position a calf for delivery	No	6.7	61.0	39.0	
Provides supplemental oxygen for calves experiencing a difficult birth	Yes	1.2	93.0	7.0	0.087
of the second of	No	98.8	80.8	19.2	
oses a source or near during cold weather for calves experiencing dystocia	Yes	50.4	84.0	16.0	0.067
Tries to elicit a suckle response for calves experiencing dust sais	ONT N	49.0	8.77	2.77	
a control of the cont	No	51.8	7.02.7	16.3	0.074
Dystocia rate for heifers in the last 12 mo	<15%	35.5	78.5	21.5	0.380
	>15%	64.5	81.7	18.3	
Lystocia rate for cows in the last 12 mo	%1>	29.5	80.4	19.6	0.884
		101	0 10	4	

Table 3. Chi-square analysis of calf-level factors for association with failure of passive transfer on US dairies

				of calves by gG level	
Calf-level variable	Level	Calves (%)	≥10 mg/mL (adequate)	<10 mg/mL (failure)	χ^2 <i>P</i> -value
All calves		100.0	80.8	19.2	
Colostrum acquisition	Nursing	25.3	74.2	25.8	0.014
ere record out of the control of th	Hand-fed	74.7	83.0	17.0	
Age in days of calf when blood collected	1	15.1	79.1	20.9	0.390
	2	17.8	82.8	17.2	
	3	14.6	83.7	16.3	
	4	12.1	82.9	17.1	
	5	14.7	84.3	15.7	
	6	11.8	71.5	28.5	
	7	13.9	79.6	20.4	
Season when calf tested	Winter (Feb to March)	12.1	81.3	18.7	0.952
	Spring (April to May)	38.8	81.4	18.6	
	Summer (June to August)	49.1	80.3	19.7	

unable to position a calf for delivery (P=0.006), whether or not the operation used a heat source for calves experiencing dystocia (P=0.067), whether or not the operation would try to elicit a suckle response for calves experiencing dystocia (P=0.074), whether or not the operation provided oxygen for calves experiencing dystocia (P=0.087), whether or not the operation had a system for scoring calving difficulty (P=0.093), and whether or not the operation pooled colostrum (P=0.273). Herd-level factors not associated with FPT in the univariable analysis included herd dystocia rates, herd size, region, storing colostrum, and pasteurizing colostrum.

Calf-Level Variables and Univariable Associations

About one-quarter (25.3%) of calves obtained colostrum by nursing and the remaining calves were handfed (Table 3). Of calves that were hand-fed, 68.7% were fed less than 3.78 L of colostrum at the first feeding (Table 4). The majority of the hand-fed calves (82.5%) received colostrum by bottle; only 13.9% of these calves received colostrum by esophageal tube (Table 4).

The calf-level variables associated (P < 0.3) with FPT in the univariable analysis were nursing (P = 0.014) and quantity of colostrum at calf's first feeding (P = 0.193) (Tables 3 and 4). Calves fed colostrum by bottle were no more likely to have FPT than calves fed by esophageal tube (P = 0.340; Table 4).

Multivariable Analysis of Herd-Level Variables

In the herd-level multivariable model, 5 herd-level variables were associated with FPT (Table 5). No interactions were statistically significant. Calves on operations that pooled colostrum were 2.2 times more likely to have FPT, and calves on operations that did not routinely monitor serum proteins in heifer calves were 13.8 times more likely to have FPT. Compared with operations that did not allow nursing and handfed colostrum to calves within 4 h of birth, the odds of FPT were 2.4 times higher for calves on operations that allowed nursing and 2.7 times higher for calves on operations that did not allow nursing and hand-fed colostrum more than 4 h after birth. Two factors relating to the calving process were associated with FPT.

Table 4. Chi-square analysis of calf-level factors for association with failure of passive transfer on US dairies that hand-fed colostrum to heifer calves

			Percentage of calves by serum IgG level		
Calf-level variable	Level	Hand-fed calves (%)	≥10 mg/mL (adequate)	<10 mg/mL (failure)	χ^2 <i>P</i> -value
All hand-fed calves (calves that did not nurse)	8	100.0	83.0	17.0	
Quantity of colostrum at calf's first feeding	< 3.78 L	68.7	81.5	18.5	0.193
•	>3.78 L	31.3	86.4	13.6	
Method of calf's first colostrum feeding	By bottle	82.5	84.2	15.8	0.340
	By tube	13.9	78.7	21.3	
	Other method ¹	3.6	69.1	30.9	

¹Category includes calves fed by bucket/pail and calves fed by more than one method.

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The odds of FPT were 2.6 times higher for calves on operations that would not call a veterinarian for assistance when they were unable to correctly position a calf for delivery and 1.6 times higher for calves on operations that did not provide a source of heat during cold weather for calves experiencing dystocia. Although herd size was not significant in the final model, model estimates for other variables changed by more than 10% when herd size was included, so it was kept in the final model to control for confounding.

Multivariable Analysis of Calf-Level Variables for Calves Hand-Fed Colostrum

In the absence of the effect of nursing, none of the calf-level variables showed an association (P < 0.05) with FPT in the calf-level multivariable model.

DISCUSSION

The prevalence of FPT in US dairy heifer calves has decreased from over 40% in 1991–1992 (USDA, 1993) to 19.2% in 2007. This apparent decrease in prevalence could have been due to changes in management practices, but differences in methodologies between the 2 studies should also be considered. Both studies measured heifer calf serum IgG using RID. Calves in the 1991–1992 study were between 24 and 48 h of age, and calves in the current study were between 1 and 7 d of age. The age difference is unlikely to influence the prevalence estimates, as calf age was not significantly associated with FPT in the current study (Table 3). In 2007, only healthy calves that had received colostrum were tested, but no such stipulation about calf health or colostrum administration was used in the 1991–1992 study. This methodology difference may explain part of the apparent decrease in FPT prevalence.

Nonetheless, there is evidence that producers are improving their colostrum management programs at least in part because of educational campaigns conveying the importance of colostrum management. For instance, dairy producers have been encouraged to separate calves from their dams as soon as possible and to provide colostrum by bottle or tube (BAMN, 2001). Published national population estimates report that the percentage of operations removing calves from the dam before nursing increased significantly from 28.0% in 1991 to 55.9% in 2007 (USDA, 2008). Educational materials have also discouraged pooling of colostrum (BAMN, 2001), and a slight decrease (27.0 to 21.0%) was seen in the percentage of dairy operations that pooled colostrum between 2002 and 2007 (USDA, 2008).

In the present study, both colostrum pooling and nursing were associated with FPT. Weaver et al. (2000)

5. Multivariable analysis of herd-level management factors for association with failure of passive transfer on US dairies

Herd-level management practice	Level	Odds ratio ¹	95% Confidence interval	Final model P -value
Routinely monitors serum proteins in newborn heifer calves	Yes No	Referent 13.82	3.60-53.07	<0.001
Seeks veterinary assistance for difficult deliveries when unable to correctly	Yes	Referent	E A	i i
position a call for delivery	No	2.02	1.19-5.76	0.017
Pools colostrum	Yes	2.20	1.24 - 3.90	0.007
	No	Referent		
Usual time of first colostrum feeding	Unknown (allowed to nurse)	2.42	1.48 - 3.96	<0.001
	>4 h after birth	2.65	1.51 - 4.66	0.001
	≤4 h after birth	Referent		
Uses a source of heat during cold weather for calves experiencing dystocia	Yes	Referent		
	No	1.55	1.01 - 2.37	0.044
Herd size (number of cows)	<100	0.75	0.43 - 1.33	0.325
	100 to 499	0.65	0.35 - 1.22	0.178
	>500	Referent		

Odds of calf having failure of passive transfer.

discouraged pooling of colostrum because immunoglobulins in the pooled colostrum may be diluted by contributing portions with high volume but low immunoglobulin levels. In addition, pooling can increase calf exposure to colostrum-borne pathogens (Godden, 2008). To the authors' knowledge, the present study is the first to demonstrate a statistical association between colostrum pooling and FPT in calves.

The increased risk of FPT in calves allowed to obtain colostrum via nursing agrees with previous research, which suggests calves left with the cow for nursing have high rates of FPT—42% in one study (Brignole and Stott, 1980) and 61% in another (Besser et al., 1991). Nursing may be associated with FPT because the calf may ingest an inadequate quantity of colostrum or because suckling may not occur in a timely fashion. Edwards and Broom (1979) observed calves after birth to determine the elapsed time before suckling. Eleven percent and 46% of calves born to first-parity dams and second-parity or older dams, respectively, did not suckle during the first 6 h after birth. Also, calves allowed to nurse can ingest manure and bacteria from the environment while searching for teats and suckling colostrum. Increased bacterial levels in colostrum have been shown to interfere with the absorption of immunoglobulins (Poulsen et al., 2002).

Calves fed colostrum more than 4 h after birth were more likely to have FPT than those fed within 4 h of birth. This is in agreement with previous literature that suggests that immunoglobulin absorption by the calf is most efficient in the first 4 h after birth and declines rapidly after 12 h of age (Weaver et al., 2000; Chigerwe et al., 2008; Godden, 2008).

Johnson et al. (2007) suggested that pasteurization of colostrum improves IgG absorption by decreasing bacterial interference with passive transfer, but the present study failed to demonstrate an association between FPT and colostrum pasteurization. Only 0.8% of calves in this study were on operations that pasteurized colostrum, so this was not a common practice, which may have precluded us from finding a significant association. Also, data were not collected about specific pasteurization protocols on these operations nor were pasteurized samples collected. Bacterial contamination of colostrum can occur if improper pasteurization techniques are used or if colostrum is stored at room temperature after pasteurization. Bacterial counts increase rapidly when colostrum is stored without refrigeration (Stewart et al., 2005). Preliminary research using a commercial batch pasteurizer suggests that on-farm pasteurization is effective in reducing bacterial contamination while preserving the IgG concentration of colostrum (Godden et al., 2006; McMartin et al., 2006). Educational materials are available to assist producers in improving on-farm pasteurization techniques (BAMN, 2008a,b). Further research on the benefits and practicality of feeding pasteurized colostrum would be helpful.

Chigerwe et al. (2008) demonstrated an association between the quantity of colostrum fed and calf IgG level. Similarly, Trotz-Williams et al. (2008) showed that feeding a higher volume of colostrum was associated with a decreased risk of FPT in Ontario dairy calves. This study did not demonstrate a significant association between FPT and quantity of colostrum fed at first feeding (<3.78 L of colostrum vs. 3.78 L or more). One potential reason for not finding a significant association may have been that the amount of colostrum at first feeding was recorded rather than the total amount of colostrum administered. Also, data were not collected about the breed or weight of each calf. The appropriate volume of colostrum for achieving successful passive transfer depends upon several factors, one of which is the weight of the calf. These issues may partially explain why colostrum quantity was not significant in this study.

A strong association was observed in the present study between FPT and the routine measurement of serum protein in heifer calves. This information should be interpreted cautiously because of a small sample size—only 3.3% of calves were on operations that routinely measured serum proteins. In addition, this association does not imply that measuring serum protein directly prevents FPT. Because these operations were routinely evaluating the passive transfer status of calves, it is logical that improving colostrum management and ultimately reducing FPT was a focus of these operations.

Two herd-level calving management factors were associated with FPT in this study. The odds of FPT were higher for calves on operations that did not typically provide a heat source during cold weather for calves experiencing dystocia. Olson et al. (1980) suggested that severe cold stress can delay or decrease the rate of absorption of immunoglobulins in dairy calves.

The odds of FPT were also higher for calves on operations that would not seek veterinary assistance when unable to correctly position a calf for delivery. A prolonged delivery can result in hypoxia and respiratory acidosis in calves (Szenci, 1983). Previous research has produced conflicting results about the relationship between acid-base balance and passive transfer. In some studies, respiratory acidosis in the calf was associated with decreased IgG absorption (Boyd, 1989; Besser et al., 1990), but other studies did not demonstrate a statistically significant relationship between IgG absorption and blood arterial partial pressure of CO₂ (Drewry et al., 1999).

It cannot be concluded from the present study that using a heat source or seeking veterinary assistance improves IgG absorption because the birth experiences of individual calves were not recorded in this study. It is likely that producers who sought veterinary assistance or provided heat sources were more focused on overall calf care, so these practices may be proxies for good general calf management practices. Calving management could be a target for future research to identify which practices are most influential in improving passive transfer.

CONCLUSIONS

The prevalence of FPT in dairy heifer calves has decreased in the last 15 yr, so progress has been made in the important area of colostrum and calf management. This study identified several management practices associated with FPT. Specifically, producers should be encouraged to hand feed single-source colostrum within 4 h of birth. In addition, calves experiencing a difficult birth may require extra attention to ensure adequate passive transfer.

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